Parameters Associated with Residual Insulin Secretion During the First Year of Disease in Children and Adolescents with Type 1 Diabetes Mellitus

R. Bonfanti*[1], E. Bazzigaluppi[2], G. Calori[3], M.C. Riva[1], M. Viscardi[1], E. Bognetti[1], F. Meschi[1], E. Bosi[2], G. Chiumello[1], E. Bonifacio[2]

[1]*Department of Paediatrics, Endocrine Unit, Scientific Institute H San Raffaele, University of Milan, Italy*
[2]*Department of Medicine, Scientific Institute H San Raffaele, University of Milan, Italy*
[3]*Epidemiology Unit, Scientific Institute H San Raffaele, University of Milan, Italy*

Factors associated with residual insulin secretion and spontaneous remission in Type 1 diabetic patients are important in the evaluation of treatment aimed at modifying the natural history of Type 1 DM. We investigated the effect of parameters at onset on residual beta cell function in 215 Type 1 DM children and adolescents. Blood gas analysis, HLA, GAD and IA-2 antibodies before the start of insulin treatment were recorded for each patient. Residual C-peptide secretion was assessed by the glucagon test, and parameters of metabolic control (HbA_{1c} and insulin dose U kg^{-1} day^{-1}) were examined at disease onset and after 3, 6, and 12 months. Residual C-peptide secretion throughout the first year of disease was significantly reduced in patients with disease onset before age 5. Multiple regression analysis showed that low pH at onset showed a significant and independent association with reduced C-peptide at 3 months ($p = 0.02$) and that the detection of GAD antibodies had a significant independent association with decreased C-peptide secretion at 6 months of follow-up ($p = 0.02$). Insulin requirement was higher in the youngest patients group and in patients with GAD antibodies. Spontaneous insulin remission (HbA_{1c} <6 % and insulin <0.3 U kg^{-1} day^{-1}) occurred in 22/192 (11 %) patients at 3 months of follow-up, in 15/190 (8 %) patients at 6 months and in 8/169 (5 %) patient at 12 months. Remission was more prevalent in older patients ($p = 0.01$) and in patients without detectable GAD antibodies: (14/64 vs 8/128, $p = 0.001$). Sex, IA-2 antibodies and HLA DR were not independently associated with C-peptide secretion, insulin requirement or remission in the first year of Type 1 DM. This study confirms the association of young age, severe acidosis at disease onset, and GAD antibodies with decreased residual beta-cell function and spontaneous remission during the first year of insulin treatment. These factors should be considered in trials evaluating therapies to retain beta-cell function and induce remission at and after disease onset. © **1998 John Wiley & Sons, Ltd.**

***Diabet. Med.* 15: 844–850 (1998)**

KEY WORDS **Type 1 diabetes mellitus; C-peptide; antibodies; remission**

Received 24 October 1997; revised 15 May 1998; accepted 7 June 1998

Introduction

Type 1 diabetes mellitus (Type 1 DM) is a result of a significant reduction of beta-cell mass. Preservation of the remaining beta-cell mass in patients at or prior to onset of clinical disease is an important goal in improving prognosis and the identification of markers which predict beta-cell function over the first year after disease onset may help to identify patients who are more likely to respond to early preventive therapy. Studies indicate that age at onset of diabetes is an important factor since younger patients show a significantly lower C-peptide secretion.[1–11] The degree of metabolic derangement and the duration of symptoms are also decribed as determinant of residual insulin secretion[3,9–11] and associations between HLA DR alleles and C-peptide secretion are reported.[6,12–13]

The pathogenesis of beta-cell destruction is likely to be mediated by autoimmunity which can be detected several years prior to clinical diabetes and a direct relationship between the level of autoimmunity in the peripheral circulation and disease risk has been demonstrated.[14,15] Several studies have tried to assess whether a relationship exists between residual beta-cell function and autoimmunity measured in the peripheral

* Correspondence to: Dr Riccardo Bonfanti, Clinica Pediatrica III, Istituto Scientifico H San Raffaele, via Olgettina 60, I-20132 Milan, Italy

 CCC 0742–3071/98/100844–07$17.50

circulation. Studies assessing the relationship between residual beta-cell function and the humoral autoimmune markers ICA or insulin antibodies give inconsistent and conflicting results.[4,6,9,12,13] Studies with respect to the humoral markers GAD and IA-2 antibodies are few. Reports in recent onset Type 1 DM patients treated with cyclosporin[16] and in adult onset Type 1 DM patients[17] suggest that patients with undetectable GAD antibodies have a higher residual insulin secretion after the introduction of insulin treatment. There are no reports in children or concerning the relationship of residual beta-cell function and IA-2 autoantibodies.

The aim of this study was to determine whether a relationship exists between humoral autoimmunity to the Type 1 DM associated autoantigens GAD and IA-2, HLA DR alleles or clinical parameters at onset and the residual beta-cell function as measured by glucagon stimulated C-peptide responses and spontaneous insulin remission during the first year of disease in a cohort of diabetic children and adolescents.

Subjects, Materials and Methods

Subjects

We studied 215 Caucasian patients (118 M, 97 F) with a median age of 8.9 (range 1–22) years. All patients were admitted to the Paediatric Department of the San Raffaele Hospital at the onset of diabetes between 1988 and 1994. Patients began insulin therapy at the time of diagnosis and after the compensation of acidosis, they were treated with three subcutaneous insulin injections daily. Patients were instructed in self home monitoring of blood and urine glucose levels and changing insulin dosage according to the result observed, in order to maintain levels of fasting blood glucose between 4.4 and 6.7 mmol l^{-1} and postprandial blood glucose under 8.9 mmol l^{-1}. HbA_{1c} and blood gas analysis (pH and HCO_3) were recorded at hospital admission. Patients were followed at the Outpatients Department of the San Raffaele Hospital at 3, 6, and 12 months after the start of insulin therapy. Partial remission was defined as a non-diabetic HbA_{1c} level (<6 %) and an insulin requirement <0.3 Ukg^{-1} day^{-1} as stated by the IDIG group.[18] Formal consent was obtained from all the patients or their parents after a clear explanation of the aim of the study, as required by the local ethical committee.

Metabolic Parameters

Insulin requirement was expressed as Ukg^{-1} day^{-1} at the start of follow-up and at 3, 6, and 12 months of insulin therapy. Diabetic control was assessed by HbA_{1c} at diagnosis of diabetes and at each time point of follow-up. HbA_{1c} was assayed by HPLC (Diamat Biorad, Milan, Italy) with a reference value in normal subjects of 4 to 6 %.

Residual beta-cell function was assessed by determination of basal C-peptide and after intravenous injection of 1 mg of glucagon.[19] The glucagon test was performed after the compensation of acidosis at the beginning of insulin therapy and at each time point of follow-up. The test was performed between 8 am and 10 am after overnight fasting. Morning insulin dose was omitted until completion of the test. Blood samples were collected before and 6 min after intravenous injection of 1 mg of glucagon (Novo, Copenhagen, Denmark). C-peptide concentration was measured by radioimmunoassay on tubes coated with anti-C-peptide antibodies (C-peptide RIA, Tecnogenetics, Milan, Italy). The detection limit of the assay was 0.01 nmol l^{-1} and the inter-assay coefficient of variation was 8 %. A complete set of metabolic data and C-peptide levels at each time point of follow-up was obtained in 169 patients: 192 patients were studied at 3 months of follow-up, 190 patients at 6 months, and 169 patients after 12 months from the start of the insulin treatment.

Antibody Measurements

GAD and IA-2 antibodies were measured by immunobinding of ^{35}S methionine labelled *in vitro* translated recombinant full length GAD_{65} and the intracellular portion of IA-2, as previously described.[20,21] The assay threshold selected as the 99th centile of control subjects was calculated at 3 units for both GAD and IA-2 antibodies. In the second GAD antibody workshop this assay had 100 % specificity and 83 % sensitivity and in the combined antibodies workshop the GAD antibody assay had a sensitivity of 76 % and a specificity of 98 %, while the IA-2 antibody assay had a sensitivity of 61 % and a specificity of 99 %. Patients were grouped into four groups according to the humoral immunity: Group A: elevated levels of both GAD and IA-2 antibodies; Group B: elevated levels of IA-2 antibodies only; Group C elevated levels of GAD antibodies only; Group D elevated levels of neither GAD nor IA-2 antibodies.

HLA Typing

HLA DR allele typing was performed in 124/215 patients using the standard microcytotoxicity test on lymphocytes isolated from blood samples by immunomagnetic beads (Dynal A.S., Oslo, Norway).[22] Patients were grouped according to their phenotypes as HLA DR3/X, DR4/Y, DR3/4, and DRX/Y where X was an allele other than DR4 and Y an allele other than DR3. Those who were HLA typed had a similar age (9.13 ± 4.4 vs 8.3 ± 4.2 years) and sex distribution (70 M/54 F vs 48 M/43 F) to those who were not typed.

Statistical Analysis

As variables were not normally distributed, non-parametric tests were used. Comparisons at each time point between variables were performed using Kruskal–Wallis analysis or Mann–Whitney U test. *Post hoc* comparisons

were adjusted by Bonferroni's procedure. Relationship among variables was investigated by Spearman correlation coefficients. The relationship between remission and antibodies was evaluated by contingency table chi-square or chi-square for trend. As comparisons were conducted at each follow-up time point, all significance tests were conducted at the alpha = 0.02 level and were two-tailed. The relationships between parameters at onset and C-peptide at 3, 6, and 12 months were evaluated by multiple regression analysis after rank transformation of the dependent variable. The statistical analysis was performed with the SAS statistical program on a personal computer.

Results

Age and Sex

Residual C-peptide secretion was significantly related to the age at onset of the patient. Figure 1 shows residual C-peptide secretion by interquartile age ranges of patients (0–5 years, 5.1–9 years, 9.1–12 years, 12.1–22 years). C-peptide secretion at each time-point of follow-up was lowest for patients in the youngest age group ($p = 0.001$). While stimulated C-peptide levels increased substantially 3 months after onset in patients with disease onset after 5 years of age, levels in the youngest age group were only marginally increased with respect to levels at onset. Thereafter levels reduced similarly in all patients regardless of age. There were no differences between males and females in C-peptide secretion during the first year after disease onset.

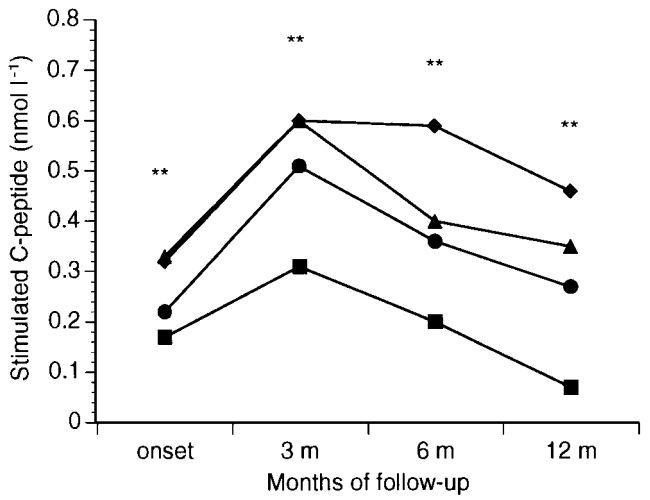

Figure 1. Residual beta cell function as measured by stimulated C-peptide secretion (nmol l^{-1}) in the first year after diabetes onset. Time of follow-up is shown on the abscissa and the median C-peptide levels on the ordinate. Patients are divided by age: ■ 0–5 yr, ● 5.1–9 yr, ▲ 9.1–12 yr, ◆ 12.1–22 yr; $^{**}p < 0.001$, 0–5 yr vs remainder, Kruskal-Wallis test

Acidosis at Type 1 DM Onset

The degree of metabolic derangement was evaluated by pH and HCO_3^-: 50 % of the patients presented with pH < 7.3 and 10 % of the patients had a severe acidosis with a pH< 7.0. The degree of acidosis at onset was significantly related to the C-peptide secretion at onset of Type 1 DM ($r = 0.22$, $p = 0.007$) and after 3 months of insulin therapy ($r = 0.23$, $p = 0.007$), but not thereafter. The relationship between acidosis and C-peptide secretion at 3 and 6 months remained even after adjustment for age. Younger children more often presented with severe acidosis at onset and there was a correlation between age and pH value ($r = 0.17$; $p = 0.016$).

Immunogenetic Parameters

Elevated GAD antibodies were found in 143/215 (66.5 %) patients at onset of disease and elevated IA-2 antibodies in 134/213 (63 %) patients. Elevated levels of at least 1 antibody were found in 192 (90 %) patients; 83 (39 %) had both, 50 (23 %) only GAD antibodies, and 59 (28 %) only IA-2 antibodies. There was no significant relationship between antibody levels and age in this relatively young cohort.

Patients with elevated GAD antibodies had a lower stimulated C-peptide secretion at 3 months of follow-up than those with undetectable levels of antibodies: 0.45 (range 0.1–1.6) vs 0.57 (range 0.12–1.33) nmol l^{-1}, $p = 0.02$; Figure 2(a). Patients with elevated IA-2 antibodies showed a higher stimulated insulin secretion at onset of diabetes than those with undetectable levels of antibodies: 0.29 (range 0.03–1.03) vs 0.22 (range 0.01–0.9) nmol l^{-1}, $p = 0.02$; Figure 2(b). Patients with elevated GAD antibodies but undetectable levels of IA-2 antibodies showed the lowest C-peptide secretion at onset ($p < 0.001$; Figure 2(c)). At 12 months of follow-up C-peptide levels were reduced in most of the patients and no differences were detected between groups.

In 124 patients studied for HLA, 63 (50 %) had HLA DR3, and 60 (48 %) HLA DR4; 23 (18 %) had both DR3 and DR4 and 24 (19 %) had neither allele. There was no significant relationship between HLA DR phenotypes and C-peptide secretion at each time point of follow-up. Patients who were not HLA typed had similar C-peptide secretion to those who were typed.

HbA_{1c} and Insulin Requirement

HbA_{1c} values at onset were lowest in patients within the youngest interquartile age group: 10.6 (age 0–5) vs 11.9 (age 5.1–9), 11.8 (age 9.1–12), 11.5 % (age >12, $p = 0.0003$). Insulin requirement in the youngest patients was higher at each time point of follow-up with a similar metabolic control (Table 1). Insulin requirement at each time point of follow-up was also higher in patients with elevated GAD antibodies than those with undetectable

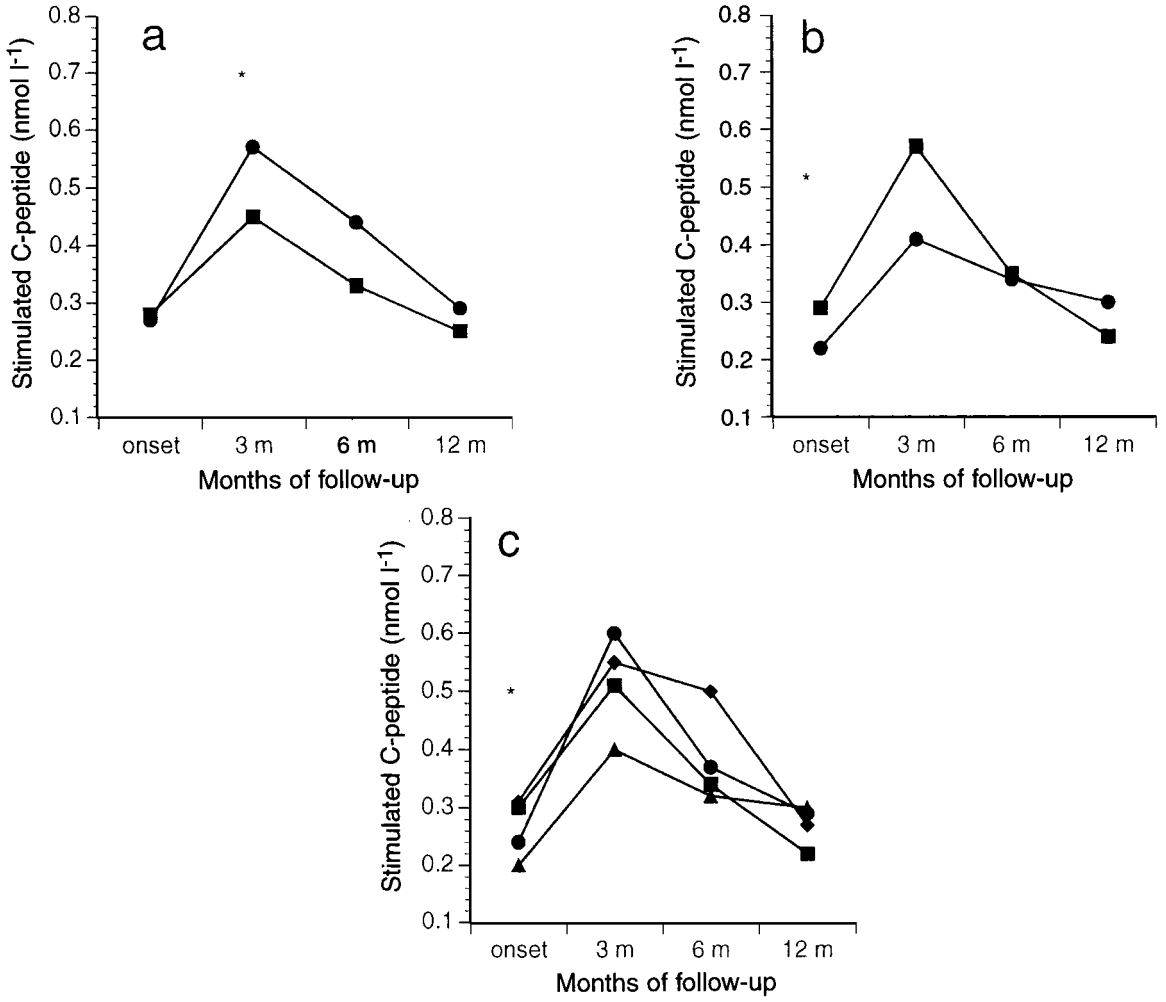

Figure 2. Residual beta cell function as measured by stimulated C-peptide secretion (nmol l^{-1}) in the first year after diabetes onset, with respect to humoral autoimmunity. Time of follow-up is shown on the abscissa and the median C-peptide levels on the ordinate.
(a) Patients with elevated ■ and undetectable ● GAD antibodies, *$p = 0.02$, Mann–Whitney U test
(b) Patients with elevated ■ and undetectable ● IA-2 antibodies, *$p = 0.02$, Mann–Whitney U test
(c) Patients with elevated GAD and IA-2 antibodies ■; patients with elevated IA-2 but not GAD antibodies ●; patients with elevated GAD but not IA-2 antibodies ▲ and patients with undetectable GAD and IA-2 antibodies ◆; *$p = 0.001$, elevated GAD but not IA-2 antibodies vs remainder, Kruskal-Wallis test

Table 1. Insulin requirement (U kg^{-1} day^{-1}) during follow-up

	Patient age			
	0–5	5.1–9	9.1–12	12.1–22
Onset	0.7	0.5	0.62	0.64
3 months	0.57[a]	0.32	0.39	0.35
6 months	0.62[a]	0.43	0.49	0.34
12 months	0.74[a]	0.61	0.71	0.51

[a]$p < 0.001$, 0–5 yr vs remainder.

GAD antibody, despite having similar HbA_{1c} levels (Table 2). HbA_{1c} and insulin requirement did not differ with respect to sex, HLA DR, degree of acidosis and IA-2 antibody levels.

Table 2. Insulin requirement and metabolic control (HbA_{1c}) between patients with and without detectable GAD antibodies

	GAD>3 units	GAD<3 units
Insulin (U kg^{-1} day^{-1})		
Onset	0.66	0.64
3 months	0.46	0.34[a]
6 months	0.5	0.42[b]
12 months	0.66	0.56[a]
HbA_{1c} (%)		
Onset	11.6	11.4
3 months	6.8	6.4
6 months	7.1	7.1
12 months	7.8	7.4

[a]$p = 0.01$, GAD > 3 units vs GAD < 3 units.
[b]$p = 0.001$, GAD > 3 units vs GAD < 3 units.

Remission

Spontaneous remission occurred in 22/192 (11 %) patients at 3 months of follow-up, in 15/190 (8 %) patients at 6 months, and in 8/169 (5 %) patients at 12 months. Remission at 3 months of follow-up was less frequent in the younger patient group: 0/42 ≤ 5 years, vs 10/63 (16 %) 5.1–9 years, vs 2/43 (5 %) 9.1–12 years, vs 10/44 (23 %) >12 years (chi-square for trend $p = 0.01$). Remission at 3 months was also less frequent in patients with elevated GAD antibodies levels: 8/128 (6 %) vs 14/64 (22 %) without GAD antibodies ($p = 0.001$). There were no differences in the frequencies of remissions with respect to sex, HLA, and IA-2 antibody levels.

Multiple Regression Analysis

In the multiple regression analysis only age showed a significant relationship to C-peptide secretion at each time point of observation ($p < 0.001$) with partial r^2 ranging from 0.15 at 3 months to 0.20 at 6 months to 0.32 at 12 months of follow-up. Low pH at onset showed a significant and independent association with reduced C-peptide at 3 months and not thereafter (partial $r^2 = 0.04$, $p = 0.02$) and elevated GAD antibodies had a significant independent association with decreased C-peptide secretion at 6 (partial $r^2 = 0.03$, $p = 0.02$) months of follow-up. IA-2 antibody levels and sex did not show independent effects on stimulated C-peptide secretion.

Discussion

Residual beta-cell function and clinical remission are parameters used in evaluating the performance of therapies in ameliorating islet beta-cell function after the onset of Type 1 DM. In this study we have examined which intrinsic factors may influence these parameters in the first year after disease onset in a cohort of Type 1 DM children and adolescents. We confirmed that young age and increased acidosis were associated with poor residual beta-cell function and infrequent remission. We also found that this was true for patients with increased levels of GAD antibodies. Other factors including sex, HLA DR, and IA-2 antibodies were not found to influence either residual beta-cell function or remission independently in this cohort. These findings should be considered when recruiting and randomizing cases within clinical trials at and after disease onset.

A relationship of C-peptide secretion with age of Type 1 DM onset has been well established.[5,7,8] This appears to be strongest when onset is in children less than 5 years of age. The explanation for the reduced insulin secretory capacity in these young patients is not entirely clear. Very young individuals will have a reduced beta-cell mass and therefore less overall beta-cell destruction is likely to be required for insulin insufficiently. It may also be postulated that there is a more aggressive beta-cell destruction in young onset cases leading to both the early disease onset and a reduced residual beta-cell function as compared to older onset patients. The youngest patients also had a more pronounced acidosis accompanying disease onset, which may also suggest a rapid deterioration of beta-cell function close to disease onset. A more pronounced acidosis was furthermore associated with a significantly reduced stimulated C-peptide secretion at, and early after, disease onset, independent of the effect of age. This latter finding is in accordance with studies of Kumulainen,[11] while studies of Sochett could not show such a relationship.[3]

In this cohort, using a limited number of patients in whom both stimulated C-peptide levels and HLA DR typing were available, we were unable to confirm previous reports[13] of reduced C-peptide levels in patients with the HLA DR3/4 phenotype, or a relationship between HLA DR and metabolic control. Relationships between HLA DR and beta cell function have been inconsistent.[12]

A relationship between humoral autoimmunity and residual beta-cell function has been sought by several groups. With respect to ICA and IAA, results have been inconsistent.[3,9–13] Two studies have reported the relationship with GAD antibodies. Petersen *et al.*, in a study performed in 71 Type 1 DM adults with a mean age of 21 years and treated with cyclosporin found significantly increased stimulated C-peptide levels 9–15 months after follow-up in patients without detectable GAD antibodies compare to those with GAD antibodies.[16] Patients without detectable antibodies also had a 20 % lower insulin requirement but there were no differences in remission between those with and without detectable GAD antibodies. In a second study in adult Type 1 DM patients, it was found that the detection of GAD antibodies was associated with a more rapid decline of beta-cell function than those without GAD antibodies.[17] Our data in children and adolescents show that patients with GAD antibodies had reduced stimulated C-peptide levels in the first 6 months after disease onset when compared to patients without detectable GAD antibodies. No differences could be found after 12 months, where, unlike in adults, C-peptide levels were low in most of the patients. A reduced residual beta-cell function in patients with GAD antibodies was also evidenced by an increased insulin requirement in these patients in order to maintain a metabolic control similar to that of patients without detectable GAD antibodies. Finally, remission was less frequent in patients with GAD antibodies, possibly as a result of the reduced insulin reserve.

An explanation for the association of GAD antibodies with stimulated C-peptide level is unclear. It may be speculated that autoimmunity against GAD and other autoantigens is associated with ongoing or aggressive beta-cell destruction. However, we know that autoantibodies occur very early in life[23] and can be detected many years prior to clinical onset of Type 1 DM, often without a detectable reduction in beta-cell function. Furthermore, there was no association between C-peptide levels and another humoral marker of Type 1 DM, IA-2

antibodies, nor was the additional detection of IA-2 antibodies associated with a further reduction of beta-cell function. Indeed, those patients with IA-2 antibodies in the absence of GAD antibodies had the highest C-peptide levels, suggesting that the residual beta-cell function is not associated with general humoral autoimmunity. Therefore, while the antibody measurements can serve as markers of risk, and possibly of prognosis, they may not correlate with pathogenesis and beta-cell destruction. Moreover it should be considered that antibody levels at onset of clinical disease may not always reflect those in the years prior to onset.

Spontaneous insulin remission is relatively infrequent during the first year of diabetes. It is in part related to residual insulin secretion and in part related to differences in insulin sensitivity present during the first year of insulin treatment, such that there is no linear relationship between the degree of residual beta cell function and remission.[24] Many factors may be determinants of spontaneous remission. These include age of onset, degree of metabolic derangement, residual C-peptide secretion, and humoral autoimmunity.[3,6,11,12,16] In our cohort of patients we were able to confirm (using a definition of partial remission with normal HbA_{1c} and insulin requirement less than 0.3 U kg^{-1} day^{-1}) the effect of age. No patients with onset of disease prior to 5 years of age had a spontaneous remission. Moreover we confirmed the effect of metabolic control at onset of Type 1 DM on the percentage of spontaneous remission, as recently shown in a paper from the Finnish group.[11] For the first time we have also found a difference in remission between patients with and without GAD antibodies.

In summary, we have confirmed, in a cohort of children and adolescent Type 1 DM patients, the important effect of age, degree of metabolic derangement, and GAD humoral autoimmunity at onset of diabetes in the determination of residual beta-cell function and spontaneous remission during the first year of follow-up. Since measurements of residual beta-cell function and spontaneous remission are considered in the evaluation of therapies aimed at maintaining beta-cell function after onset of Type 1 DM, there may be an indication to include these parameters in the recruitment and randomization of patients in clinical trials and in the interpretation of outcome.

References

1. Madsbad S. Prevalence of residual beta cell function and its metabolic consequences in Type 1 diabetes. *Diabetologia* 1983; **24**: 141–147.
2. Madsbad S, Krarup T, Regeur L, Faber OK, Binder C. Insulin secretion reserve in insulin dependent patients at the time of diagnosis and the first 180 days of insulin treatment. *Acta Endocrinol* 1980; **95**: 359–363.
3. Sochett EB, Daneman D, Clarson C, Ehrlich RM. Factors affecting and patterns of residual insulin secretion during the first year of type 1 (insulin-dependent) diabetes mellitus in children. *Diabetologia* 1987; **30**: 453–459.
4. Schiffrin A, Suissa S, Poussier P, Guttmann P, Weitzner G. Prospective study of predictors of β cell survival in Type 1 diabetes. *Diabetes* 1988; **37**: 920–924.
5. The DCCT Research Group. Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual β-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endocrinol Metab* 1987; **65**: 30–36.
6. Martin S, Pawlowski B, Greulich B, Ziegler A, Mandrup-Poulsen T, Mahon J. Natural course of remission in IDDM during 1st yr after diagnosis. *Diabetes Care* 1992; **15**: 66–74.
7. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M. A comparison of childhood and adult type 1 diabetes mellitus. *New Engl J Med* 1989; **320**: 881–886.
8. Montana E, Fernandez-Castaner M, Rosel P, Gomez J, Soler J. Age, sex and ICA influence on beta-cell secretion during the first year after the diagnosis of type 1 diabetes mellitus. *Diabete Metabolisme* 1991; **17**: 460–468.
9. Couper JJ, Hudson I, Werther GA, Warne GL, Court JM, Harrison LC. Factors predicting residual beta cell function in the first year after diagnosis of childhood type 1 diabetes. *Diabetes Res Clin Pract* 1991; **11**: 9–16.
10. Pinkney JH, Bingley PJ, Sawtell PA, Dunger DB, Gale EAM. Presentation and progress of childhood diabetes mellitus: a prospective population based study. *Diabetologia* 1994; **37**: 70–74.
11. Komulainen J, Lounamma R, Knip M, Kaprio E, Akerblom H. Ketoacidosis at the diagnosis of type 1 (insulin-dependent) diabetes mellitus is related to poor residual β cell function. *Arch Dis Child* 1996; **75**: 410–415.
12. Mandrup-Poulsen T, Molvig J, Andersen HU, Helquist S, Spinas G, Munck M. Lack of predictive value of islet cell antibodies, insulin antibodies and HLA DR phenotypes for remission in cyclosporin treated IDDM patients. *Diabetes* 1990; **39**: 204–210.
13. Ludgvisson J, Safwenberg J, Heding LG. HLA types, C-peptide and insulin antibodies in juvenile diabetes. *Diabetologia* 1977; **13**: 13–17.
14. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, *et al*. Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 1994; **43**: 1304–1310.
15. Seissler J, Morghenthaler NG, Achenbach P, Lampeter EF, Glawe D, Payton M, *et al*. Combined screening for autoantibodies to IA-2 and antibodies to glutammic acid decarboxylase in first degree relatives of patients with IDDM. *Diabetologia* 1996; **39**: 1351–1356.
16. Petersen J, Dryberg T, Karsen A, Molvig J, Michelsen B, Nerup J, Mandrup-Poulsen T. Glutamic acid decarboxylase (GAD 65) autoantibodies in prediction of β-cell function and remission in recent onset IDDM after cyclosporin treatment. *Diabetes* 1994; **43**: 1291–1296.
17. Gootsater A, Ladin-Olsson M, Lernmark A, Ferlund P, Sundkwist G, Hagopian WA. Glutamate decarboxylase antibody levels predict rate of β-cell decline in adult-onset diabetes. *Diabetes Res Clin Pract* 1995; **27**: 133–140.
18. Pozzilli P. Immunotherapy in type 1 diabetes. *Diabetic Med* 1988; **5**: 734–738.
19. Faber OK, Binder C. C-peptide response to glucagon: a test for residual β-cell function in diabetes mellitus. *Diabetes* 1977; **26**: 605–610.
20. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin dependent diabetes related 37/40 K autoantigen and a target of islet cell antibodies. *J Immunol* 1995; **155**: 5419–5426.

21. Schmidli RS, Colman PG, Bonifacio E and Participating Laboratories. Disease sensitivity and specificity of 52 assays for glutammic acid decarboxilase antibodies. The Second International GADAb Workshop. *Diabetes* 1995; **44**: 636–640.
22. Teresaki PI, Bernoro D, Park MS, Ozturk G, Ikaki Y. Microdroplet testing for HLA A, B, C and D antigens. *Am J Clin Path* 1978; **69**: 103–120.
23. Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hawkes CJ, Ziegler AG. Perinatal autoimmunity in offspring of diabetic parents: the German multicenter BABY-DIAB study. Detection of humoral immune response to islet autoantigens in early childhood. *Diabetes* 1996; **45**: 967–973.
24. Amiel SA, Scherwin R, Simonson D, Lauritano A, Tamborlane W. Impaired insulin action in puberty, a contributing factor to poor glycaemic control in adolescent with diabetes. *N Engl J Med* 1986; **315**: 215–219.